

ORIGINAL ARTICLE

Cow's milk allergy and neonatal short bowel syndrome: comorbidity or true association?

A Diamanti¹, AG Fiocchi², T Capriati^{1,3}, F Panetta¹, N Pucci⁴, F Bellucci¹ and G Torre¹

BACKGROUND/OBJECTIVES: Neonatal short bowel syndrome (SBS) follows early intestinal resections that may expose the children to increased intestinal contact with undigested food proteins and to the risk of food allergy. We report three consecutive cases of cow's milk allergy (CMA) in SBS infants.

SUBJECTS/METHODS: We reviewed three cases of CMA developed in 37 children with neonatal SBS followed up in the last 10 years. The setting of the survey was the Gastroenterology-Hepatology and Nutrition Unit of the Pediatric Hospital 'Bambino Gesù' in Rome. The diagnosis of CMA was based on the oral food challenge and was supported by the results of the skin prick tests (SPT) and/or the specific immunoglobulin (Ig) E.

RESULTS: Two patients had persistent liquid stools and periodic episodes of vomiting when they were fed with an intact milk protein-based formula, that disappeared with extensively hydrolyzed formula and amino-acid-based formulae, respectively. The third patient developed maculo-papular rash, flushing and angioedema, when he was introduced a regular formula. The challenge-confirmed CMA in all patients. Positive specific IgE for milk proteins was documented in all the three patients. Two out of the three patients had positive familial history for allergy and positive SPT.

CONCLUSIONS: Our findings suggest that the SBS patients require a careful clinical monitoring of the tolerance for the cow's milk proteins, because CMA could be more frequent than expected. A prospective regular assessment for the potential cow milk sensitization by SPT and specific IgE may clarify the nature of the association and support the clinical surveillance. Multicenter studies are required to better evaluate this comorbidity.

European Journal of Clinical Nutrition advance online publication, 3 September 2014; doi:10.1038/ejcn.2014.156

INTRODUCTION

Neonatal short bowel syndrome (SBS) follows early intestinal resections that may expose the SBS children to increased intestinal contact with undigested food proteins and, as consequence, to the risk of food allergy.^{1,2} Few studies specifically address this potential comorbidity.^{3,4} We report here three consecutive cases of cow's milk allergy (CMA) in patients with neonatal SBS and we discuss the relationship between the two diseases.

PATIENTS AND METHODS

We reviewed the cases of CMA that developed in three children with SBS at neonatal onset. During the past 10 years (2004–2013) in our institution we have completed the clinical follow-up, from the first surgery to the complete introduction of a diversified diet including dairy products, in 37 patients with neonatal SBS, with protracted intestinal failure and malabsorption, requiring caloric intake by parenteral nutrition (PN) $\geq 50\%$ of the energy requirements for at least 3 months (see Table 1 for the details). The setting of the survey was the Gastroenterology-Hepatology and Nutrition Unit of the Pediatric Hospital 'Bambino Gesù' in Rome. In our institution, the diagnosis of CMA, suggested by the clinical symptoms and supported by the results of the skin prick test (SPT) and/or the levels of the specific IgE for the cow's milk proteins, is based on the oral food challenge (OFC), as advised.⁵

RESULTS

Case 1

A 4-month-old boy underwent three surgical interventions for midgut volvulus in the first 2 months of life, with residual small

bowel length of 28 cm. He arrived in our center when he was 3 months old, presenting with diarrhea and periodic episodes of vomiting when fed an intact milk protein-based formula. As per protocol, he was shifted to an extensively hydrolyzed formula, but continued to show a high stool output. Therefore, when he was 4 months old, because of a suspected CMA, an amino-acid-based formula was introduced. The infant immediately showed reduced stool output and improved growth during the following days. In this patient, positive specific IgE for milk proteins was documented (cow's milk 1.06 kU/l, alpha-lactoglobulin 1.2 kU/l, beta-lactoglobulin 0 kU/l, casein 1.5 kU/l). At the age of 7 months, an OFC with a lactose-free formula containing intact cow's milk proteins was performed, and the patient developed severe diarrhea and vomiting after the 40-ml dose. The yearly follow-up challenges turned negative at 3 years, and a free diet including dairy products was successfully introduced. This patient had positive familial history for allergy.

Case 2

A 5-month-old boy had SBS owing to midgut volvulus, with a residual small bowel length of 7 cm. After two surgical interventions in the first 2 months of life, he started a nutritional rehabilitation with a regular formula, with persistent high stool output and repeated episodes of vomiting. When he arrived in our center, the boy, according to our protocol, was shifted to an extensively hydrolyzed formula, with an immediate improvement in the stool output and vomiting. After discharge, when he was

¹Artificial Nutrition Unit–Gastroenterology, Hepatology and Nutrition Unit, 'Bambino Gesù' Children's Hospital, Rome, Italy; ²Allergy Unit, 'Bambino Gesù' Children's Hospital, Rome, Italy; ³Pediatric Clinic of University, Bari, Italy and ⁴Allergy and Clinical Immunology Unit, 'Anna Meyer' Children's Hospital, Florence, Italy. Correspondence: Dr A Diamanti, Hepatology, Gastroenterology and Nutrition Unit, 'Bambino Gesù' Children Hospital, Rome 00165, Italy.

E-mail: antonella.diamanti@opbg.net

Received 4 January 2014; revised 23 June 2014; accepted 2 July 2014

5 months old, he was erroneously fed a meal of regular formula, and he immediately reacted by vomiting, diarrhea and severe dehydration. Thus, we did not challenge him at OFC. When he was 11 months old, he was also found positive at specific IgE determination with milk proteins with SPT positive at 6 mm diameter for casein and beta-lactoglobulin. The follow-up challenge at 17 months was positive at a high dose (60 ml). Thus, this 20-month-old child is now consuming up to 30 ml of

milk (or equivalent dairy products amounts) with tolerance. This patient had positive familial history for allergy.

Case 3

A 12-month-old boy, born with multiple intestinal atresia, underwent two surgical interventions in the first 2 months, with residual small bowel length of 30 cm. As per protocol, he was introduced an extensively hydrolyzed formula, followed by a complementary feeding with solid foods including cow's milk baked proteins at 6 months. When he was 12 months old, soon after his first meal with regular formula, he rapidly developed generalized maculopapular rash, flushing, angioedema and hypotension. He was treated with rehydration, steroids and anti-histaminic drugs; the extensively hydrolyzed formula was reintroduced. SPT was positive at 6 mm diameter and specific IgE for milk proteins was also positive (cow's milk 2.36 kU/l, alpha-lactoglobulin 0.00 kU/l, beta-lactoglobulin 0.00 kU/l, casein 3.35 kU/l). After 2 months, a confirmatory OFC with milk elicited maculopapular rashes and flushing at a 25-ml dose. At present, this 17-month-old child is in a good clinical condition. His diet includes small amounts of baked milk proteins. This patient had no positive familial history for allergy.

In Table 2, we report the main clinical characteristics of the three patients and the results of the biological and nutritional assessment when they developed the CMA.⁶ In the table the caloric intake provided by parenteral nutrition is reported as percent of total energy requirements by age (LARN).⁶

DISCUSSION

This report is the first published experience of challenge-confirmed CMA in SBS. A potential pitfall of this experience may be that the children with SBS often have frequent, loose-liquid

Table 1. Clinical characteristics of the series of SBS patients followed up in the last 10 years

No. of patients	37
Current age	1.25–10 years
M/F	24/13
Gestational age	27–39 weeks
Birth weight	1320–3750 g
No. of intestinal surgery	2–6
Age at the first surgery	1–7 days
<i>Underlying disease for SBS</i>	
Midgut volvulus	44%
Multiple intestinal atresia	25%
Necrotizing enterocolitis	19%
Gastroschisis	9%
Mesenteric ischemia	3%
Remaining intestinal length (range)	7–80 cm
ICV+	57%
Residual colon > 50%	84%

Abbreviations: F, female; ICV, preserved ileo-cecal valve; M, male; SBS, short bowel syndrome.

Table 2. Clinical characteristics and biological/nutritional assessment at CMA development

	Case 1	Case 2	Case 3
Sex	M	M	M
Gestational age (weeks)	39	38	32
Birth weight (g)	3260	3900	2950
Underlying disease	Volvulus	Volvulus	Atresias
Remaining bowel length (cm)	28	7	30
Remaining bowel	Jejunum-ileum	Jejunum	Ileum
Maintained ileo-cecal valve	Yes	Not	Not
Remaining colon (%)	100	50	60
Number of surgeries	1	2	2
Treatments for bacterial overgrowth	Miconazole, metronidazole probiotics	Miconazole, metronidazole, nebicine	Miconazole, metronidazole, nebicine
The time of developing CMA (months)	4	5	12
<i>Biological and nutritional assessment at developing CMA</i>			
White cell count/mm ³	10 200	5320	7980
IgG (mg%) ^a	242	279	788
IgA (mg%) ^a	57	33	5
IgM (mg%) ^a	81	66	162
IgE (mg%) ^a	21.8	18	4.5
Specific IgE for cow's milk proteins	Positive	Positive	Positive
Skin prick test for milk	Not performed	Positive	Positive
Caloric intake by PN (%) ^b	74%	80	0
Type of feeding	Oral	Oral	Oral
Weight percentile (°)	25–50	25	90–97
Height percentile (°)	50	50	75–90

Abbreviations: CMA, cow's milk allergy; Ig, immunoglobulin; PN, parental nutrition. ^aNormal reference range until 12 months for IgG 231–919 mg%, IgA 8–85 mg%, IgM 74–204 mg% and for IgE < 20 mg%. ^bIt is reported here the caloric intake provided by PN as % of the total energy requirements by age (LARN).⁶

stool that makes it difficult to evaluate whether this is only due to SBS itself or whether there is a role for an additional CMA. However, the first two patients not only had persistent liquid stools but also had repeated episodes of vomiting, which disappeared after cow's milk exclusion and relapsed after the challenge. The third patient had no gastrointestinal manifestations of CMA.

The prevalence of challenge-confirmed CMA over 37 SBS infants was therefore 8.1%, which is two- to fourfold higher than in open populations. In birth cohort study, indeed the prevalence of challenge-confirmed CMA ranges from 1.9 to 4.9%.^{7–10}

The association between CMA and SBS could be based on some specific pathophysiological characteristics of the SBS patients. First of all, a low digestive capacity may have a role in the high incidence of food allergy. As their peptic digestion is not complete during early life, protein remnants of the diet could act as allergens. Studies on the use of anti-acid drugs have clearly linked the impairment of gastric function with sensitization against oral proteins and drugs.¹¹

Only gastroenterological surgery leading to SBS is associated with a high incidence of food allergy: gastrointestinal atresia, Hirschsprung disease, congenital diaphragmatic hernia, perforation of the ileum, necrotizing enterocolitis, exomphalos¹² and hepatic transplantation surgery¹³ have been linked to CMA. It is possible that a dysfunction of the gastrointestinal tract resulting from primary diseases,¹⁴ surgical invasion, small-bowel bacterial overgrowth (SBBO) and/or atrophy in the intestinal mucosa caused by extended fasting before and after surgery have a role in CMA inception. It is commonly reported that, after small-bowel resections, the increased intestinal permeability to luminal contents relates to immune dysfunction from loss of gut-associated lymphoid tissue.^{1,13} The alterations of permeability are the prominent part of this disorder, as physiologic considerations^{2,10,15–17} and few clinical studies^{18,19} indicate. The SBBO seen in patients with SBS can in turn lead to mucosal inflammation, increased permeability to dietary antigens and sensitization to food.¹⁹

In light of the above-reported pathophysiological concerns combined with the clinical evidences of the comorbidity^{3,18,20,21} (see Table 3 for the details), the management of the SBS may include a specific strategy that takes into account the diagnosis and the prevention of CMA. Thus, the feeding approach to SBS should be aimed at preventing the development of CMA but also at optimizing the bowel adaptation for achieving the enteral autonomy.

A recent systematic review from the European Society of Allergy and Clinical Immunology for infants at risk for allergy indicates that: (1) there are mixed findings about the preventive benefits

of breastfeeding; (2) there was evidence to recommend avoiding cow's milk and substituting with extensively or partially hydrolyzed formulae for the first 4 months; and (3) it is not preventive delaying the introduction of solid foods beyond 4 months.²²

These indications should be shared with the practice regarding the SBS nutrition, which, to date, is not very standardized but more 'experience based' rather than 'evidence based', partly owing to the small number of patients with this condition.²²

The hydrolyzed formulas, if the breast milk is not available, are the most commonly used and are well tolerated by most patients.^{2,19,23–28}

Human milk contains nucleotides, immunoglobulin (Ig) A and leukocytes, which are shown to support the neonate's immune system.^{29,30} Human milk also contains glutamine and growth factors, such as growth hormone and epidermal growth factor, which possibly promote bowel adaptation.^{31,32} Andorsky *et al.*³³ found that the use of breast milk has the highest correlation with shorter PN courses.

Only one study prospectively investigated the role of hydrolyzed formula compared with a whole-protein formula in promoting growth and development in SBS. The authors found no significant differences between the two treatments in terms of weight gain, enteral feed tolerance and energy expenditure. All patients in this study, nevertheless, were on PN and they were evaluated for a too short period to judge the effects of the two formulae on the course of intestinal adaptation.³⁴ Unlike this report, a previous survey found that amino-acid-based formulae are associated with a shorter duration of PN.³³ Even before this study, Bines *et al.*²⁰ reported that four patients who had SBS and feeding intolerance were all weaned off PN within 15 months of being started on elemental diets.

Physiologic data, nevertheless, fail to support the use of amino-acid-based formulae in SBS. Evaluation of specific macronutrients reveals that hydrolyzed protein is more trophic to the gut than intact protein.¹ It has also been clearly shown that elemental diets containing amino acids may not be as effective as hydrolyzed formulae in maximizing adaptation.^{35,36} Monosaccharides requiring no digestion, induce little mucosal hyperplasia as compared with disaccharides.²

In a recent review, Goulet *et al.* suggested three steps for the feeding of SBS infants to optimize the bowel adaptation: (1) breast milk or whole-protein formula, as the first choice; (2) extensively hydrolyzed formula, if the former are not tolerated; and (3) amino-acid-based formula, if there is a suspected intolerance to the previous.

We summarize in a clinical algorithm the possible managing strategy that may combine the double need of preventing the CMA, but also of enhancing the intestinal adaptation (Figure 1).

Table 3. Summary of the reports of the comorbidity SBS and CMA

Author (reference no.)	No. of pts (years)	Symptoms of CMA (formula feeding at the diagnosis)	Positive SPT/specific IgE and histology (% of the pts)	OFC with CM	Post-surgery formula	Elimination diet
Bines <i>et al.</i> ²⁰	4 (1.9–4.75)	Vomiting watery diarrhea	SPT (25%)	Not reported	eHF	AA
Mazon <i>et al.</i> ³	8 (1–7.3)	Buttock rash (eHF) No symptoms (BF and eHF)	SPT/specific IgE (100%)	Performed and positive in 2 patients	BF eHF	eHF AA
Avery Ching <i>et al.</i> ²¹	5 (0.2–13.4)	Suspected peptic disease Suspected SBBO diarrhea gastrointestinal bleeding (Not reported)	Histology (100%)	Not reported	Not reported	eHF
Masumoto <i>et al.</i> ¹⁸	2 (0.4–0.9)	Bloody stool poor general condition declined activity (CM)	Specific IgE (100%)	Not reported	AA	eHF
Diamanti (present study)	3 (0.4–1)	Vomiting watery diarrhea maculo-papular rash (2 cm, 1 eHF)	SPT/specific IgE (100%)	Performed and positive in all cases	2 CM 1 eHF	2 eHF 1 AA

Abbreviations: AA, amino-acid-based formula; BF, breastfeeding; CM, cow's milk; CMA, cow's milk allergy; eHF, extensively hydrolyzed formula; OFC, oral food challenge; pts, patients; SBBO, small-bowel bacterial overgrowth; SPT, skin prick test.

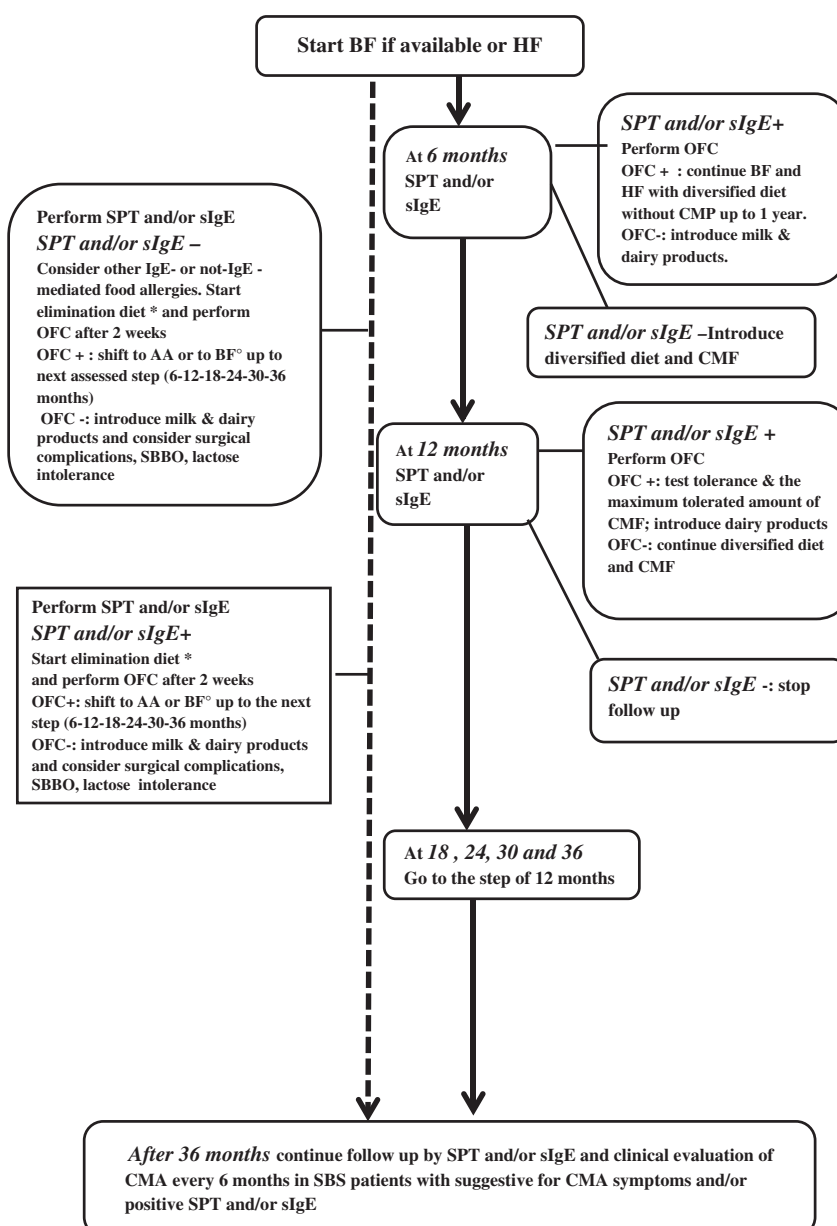


Figure 1. Dotted arrow: subjects who develop the symptoms between the steps. Complete arrow: subjects who do not show any symptoms between the steps. AA: amino-acid-based formulas; BF: breastfeeding; CMA: cow's milk allergy; CMF: cow's milk-based formulae; CMP: cow's milk protein; eHF: extensively hydrolyzed formulas; OFC: oral food challenge; SBBO: small-bowel bacterial overgrowth; SPT: skin prick tests. *Elimination diet: AA in subjects receiving HF and maternal elimination of CMP in those receiving breastfeeding. BF°: maternal elimination of CMP, integrated by calcium.

Careful attention should be reserved to SBS infants younger than 2 years, who are more prone to allergic injury than older children.¹⁹

In conclusion, our findings seem to suggest that the SBS patients require a careful clinical monitoring of the tolerance for the cow's milk proteins because CMA could be more frequent than expected. A prospective regular assessment for the potential cow's milk sensitization by SPT and specific IgE may clarify the nature of the association and support the clinical surveillance. To date, this association is suggested, nevertheless, by pathophysiological studies and by a few clinical surveys, and our experience is too small to clearly define whether it could be casual or not. Multicenter studies are therefore required to better evaluate this comorbidity.

REFERENCES

- Vanderhoof JA, Grandjean CJ, Burkley KT, Antonson DL. Effect of casein versus casein hydrolysate on mucosal adaptation following massive bowel resection in infant rats. *J Pediatr Gastroenterol Nutr* 1984; **3**: 262–267.
- Vanderhoof JA, Langnas AN. Short-bowel syndrome in children and adults. *Gastroenterology* 1997; **113**: 1767–1768.
- Mazon A, Solera E, Alentado N, Oliver F, Pamies R, Caballero L *et al*. Frequent IgE sensitization to latex, cow's milk, and egg in children with short bowel syndrome. *Pediatr Allergy Immunol* 2008; **19**: 180–183.
- Leechawengwongs E, Tison BE, Gopalakrishna GS, Reid BS, Bacino CA, Haws AL *et al*. Does short bowel syndrome increase the risk of food allergy and eosinophilic gastrointestinal disease? Observations in Shah-Waardenburg syndrome. *J Allergy Clin Immunol* 2012; **131**: 251–255.
- Fiocchi A, Brozek J, Schunemann H, Bahna SL. World Allergy Organization (WAO) Diagnosis and Rationale for Action against Cow's Milk Allergy (DRACMA) Guidelines. *World Allergy Organ J* 2010; **3**: 57–161.

- 6 LARN 1996 (Recommended Dietary Allowances for the Italian Population) from SINU (Italian Society of Human Nutrition).
- 7 Saarinen KM, Juntunen-Backman K, Jarvenpaa AL, Kuitunen P, Lope L, Renlund M et al. Supplementary feeding in maternity hospital and the risk of cow's milk allergy: A prospective study of 6209 infants. *J Allergy Clin Immunol* 1999; **104**: 457–461.
- 8 Host A, Halken S, Jacobsen HP, Christensen AE, Herskind AM, Plesner K. Clinical course of cow's milk protein allergy/intolerance and atopic diseases in childhood. *Pediatr Allergy Immunol* 2002; **13**: 23–28.
- 9 Schrandt JJ, Van Den Bogart JP, Forget PP, Schrandt-Stumpel CT, Kuijten RH, Kester AD. Cow's milk protein intolerance in infants under 1 year of age: a prospective epidemiological study. *Eur J Pediatr* 1993; **152**: 640–644.
- 10 Kvenshagen B, Halvorsen R, Jacobsen M. Adverse reactions to milk in infants. *Acta Paediatr* 2008; **97**: 196–200.
- 11 Pali-Schöll I, Jensen-Jarolim E. Anti-acid medication as a risk factor for food allergy. *Allergy* 2011; **66**: 469–477.
- 12 Miyazawa T, Itabashi K, Imai T. Retrospective multicenter survey on food-related symptoms suggestive of cow's milk allergy in NICU neonates. *Allergol Int* 2013; **62**: 85–90.
- 13 Wisniewski J, Lieberman J, Nowak-Węgrzyn A, Kerker N, Arnon R, Iyer K et al. De novo food sensitization and eosinophilic gastrointestinal disease in children post-liver transplantation. *Clin Transplant* 2012; **26**: E365–E371.
- 14 Welters CF, Dejong CH, Deutz NE, Heineman E. Intestinal adaptation in short bowel syndrome. *ANZ J Surg* 2002; **72**: 229–232.
- 15 Schrandt JJ, Van Den Bogart JP. Cow's milk protein intolerance in infants under 1 year of age: a prospective epidemiological study. *Eur J Pediatr* 1993; **152**: 640–644.
- 16 Barclay AR, Beattie LM, Weaver LT, Wilson DC. Systematic review: medical and nutritional interventions for the management of intestinal failure and its resultant complications in children. *Aliment Pharmacol Ther* 2011; **33**: 175–184.
- 17 Saylor JD, Bahna SL. Anaphylaxis to casein hydrolysate formula. *J Pediatr* 1991; **118**: 71–74.
- 18 Masumoto K, Esumi G, Teshiba R, Nagata K, Hayashida M, Nakatsujiet T et al. Cow's milk allergy in extremely short bowel syndrome: Report of two infants. *Eur J Clin Nutr Metab* 2008; **3**: e217–e219.
- 19 Vanderhoof JA, Young RJ. Hydrolyzed versus nonhydrolyzed protein diet in short bowel syndrome in children. *J Pediatr Gastroenterol Nutr* 2004; **38**: 107.
- 20 Bines J, Francis D, Hill D. Reducing parenteral requirement in children with short bowel syndrome: impact of an amino acid-based complete infant formula. *J Pediatr Gastroenterol Nutr* 1998; **26**: 123–128.
- 21 Avery Ching Y, Modia BP, Jaksica T, Duggan C. High diagnostic yield of gastrointestinal endoscopy in children with intestinal failure. *J Pediatr Surg* 2008; **43**: 906–910.
- 22 deSilva D, Geromi M, Halken S, Host A, Panesar SS, Muraro A et al. Primary prevention of food allergy in children and adults: systematic review. *Allergy* 2014; **69**: 590–601.
- 23 Olieman JF, Poley MJ, Gischler SJ, Penning C, Escher JC, van den Hoonaard TL et al. Interdisciplinary management of infantile short bowel syndrome: resource consumption, growth, and nutrition. *J Pediatr Surg* 2010; **45**: 490–498.
- 24 Goulet O, Ruemmele F, Lacaille F, Colomb V. Irreversible intestinal failure. *J Pediatr Gastroenterol Nutr* 2004; **38**: 250–269.
- 25 Vanderhoof JA, Young RJ. Enteral nutrition in short bowel syndrome. *Semin Pediatr Surg* 2001; **10**: 65–71.
- 26 Goday PS. Short bowel syndrome: how short is too short? *Clin Perinatol* 2009; **36**: 101–110.
- 27 Olieman JF, Penning C, Spoel M, Ijsselstijn H, van den Hoonaard TL, Escher JC et al. Long-term impact of infantile short bowel syndrome on nutritional status and growth. *Br J Nutr* 2012; **107**: 1489–1497.
- 28 Goulet O, Olieman J, Ksiazek J, Spolidoro J, Tibboe D, Köhler H et al. Neonatal short bowel syndrome as a model of intestinal failure: Physiological background for enteral feeding. *Clinical Nutrition* 2013; **32**: 162–171.
- 29 Cummins AG, Thompson FM. Effect of breast milk and weaning on epithelial growth of the small intestine in humans. *Gut* 2002; **51**: 748–754.
- 30 Playford RJ, Macdonald CE, Johnson WS. Colostrum and milk-derived peptide growth factors for the treatment of gastrointestinal disorders. *Am J Clin Nutr* 2000; **72**: 5–14.
- 31 Buchman AL, Scolapio J, Fryer J. AGA technical review on short bowel syndrome and intestinal transplantation. *Gastroenterology* 2003; **124**: 1111–1134.
- 32 Di Baise JK, Young RJ, Vanderhoof JA. Intestinal rehabilitation and the short bowel syndrome: part 1. *Am J Gastroenterol* 2004; **99**: 1386–1395.
- 33 Andorsky DJ, Lund DP, Lillehei CW, Jaksic T, Dicanzio J, Richardson DS et al. Nutritional and other postoperative management of neonates with short bowel syndrome correlates with clinical outcomes. *J Pediatr* 2001; **139**: 27–33.
- 34 Ksiazek J, Piena M, Kierkus J, Lyszkowska M. Hydrolyzed versus nonhydrolyzed protein diet in short bowel syndrome in children. *J Pediatr Gastroenterol Nutr* 2002; **35**: 615–618.
- 35 Fairclough PD, Hegarty JE, Silk DB, Clark ML. Comparison of the absorption of two protein hydrolysates and their effects on water and electrolyte movements in the human jejunum. *Gut* 1980; **21**: 829–834.
- 36 Poullain MG, Cezard JP, Roger L, Mendy F. Effect of whey proteins, their oligopeptide hydrolysates and free amino acid mixtures on growth and nitrogen retention in fed and starved rats. *J Parenter Enteral Nutr* 1989; **13**: 382–386.